Reviewer's report

Title: DIFFERENTIAL GENE EXPRESSION IN THE MURINE GASTRIC FUNDUS LACKING INTERSTITIAL CELLS OF CAJAL

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Reviewer: Thilo Wedel

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after discretionary revisions

The background of the present study is based on the fact that interstitial cells of Cajal (ICC) - apart from the enteric nervous system - have been recognized to be crucially important for the mediation of gastrointestinal motility. Recent histopathologic studies have shown that several motility disorders of both the upper and lower gastrointestinal tract are associated with a relative or complete loss of these pacemaker cells.

The authors have studied the gene expression profile of the gastric fundus of wild type and w/wV mice, an established animal model characterized by a relative loss of ICC. Using a microarray analysis and semiquantitative RT-PCR, 21 genes have been found to be upregulated (n=11) or downregulated (n=10) in w/wV mutants.

Although none of the genes identified have been associated previously with disorders of gastrointestinal motility, it is emphasized that the use of modern moleculargenetic technologies may allow the recognition of molecular mechanisms responsible for - or at least involved in - the loss of ICC and, thus, the impairment of the gastrointestinal transit.

COMMENTS

Introduction
The term "chronic constipation" is a rather unspecific clinical description. The patients studied in the cited paper (13) correspond to a well defined subgroup termed "slow-transit constipation".

Material and Methods
Tissue preparation: The authors should indicate whether the submucosa was left attached to the mucosa.
RNA preparation: TRIZOL is generally used to extract total RNA. However, it was stated that only poly (A)+RNA (mRNA) has been isolated by this procedure. The authors should clarify this methodologic aspect.
Semi-quantitative RT-PCR: The authors should state that the primers used for RT-PCR were derived
from different exons to exclude genomic contaminations. "G3PDH" was used as an internal control - does this housekeeping gene correspond to GAPDH?

Discussion
As the subheading "Molecular Studies of Wild Type and W/WV Mice" is not followed by another one, it should be omitted.
The sentence "It was reported that in a case of coproporphyria (CPO deficiency) family showed constipation and abnormal colic that striking features in these patients" has to be rewritten to make semantic sense.
The authors speculate on the role of CPO in the altered gastric responses of w/wv mice. Which link do they exactly postulate?
A comparison of the findings derived from the present study with results obtained from previous studies has not been carried out. However, it seems to be mandatory to cite and discuss those differential gene expression profiles which have been identified within other segments of the GIT. To which extent do they differ from the present findings and are there promising candidate genes differentially expressed in the entire GIT of this mouse model?
To their credit the authors state that none of the up- or downregulated genes within the murine gastric smooth muscle layer has been implicated in any aspect of GIT motility. Moreover, the study design does not allow to verify which of the many cell populations residing within the tunica muscularis displays the altered gene expression profiles. Nevertheless, it is emphasized that the present data generated by gene microarray analysis will "provide new insights into the molecular mechanisms responsible for the selective loss of ICC populations". Which investigations and methodologic approaches do the authors consider as helpful to clarify these mechanisms in further studies? How is it possible to differentiate between changes of gene expression pattern which are indeed responsible for the loss of ICC (causally related) and which are merely the result of compensatory effects (secondary events)?
As this study is of basic science character and was aimed to elucidate the molecular genetic aspects underlying gastrointestinal motility disorders, the readers (mainly clinicians) should be informed about the following issue: Which clinical impact in terms of diagnostic procedures and therapeutic options do the authors expect from a screening for differentially expressed genes? Is there a clinical experience available in patients characterized by severe gastrointestinal motility disorders associated with definitive alterations of ICC populations?

Figures
Layout of Fig. 1A and 1B has to be improved by correcting the position of "w/wv".

General remarks
Some formal aspects of the paper have to be improved (e.g. punctuation, orthography, consequent use of abbreviations, correct citation of references according to the guidelines of BMC Gastroenterology)

Competing interests:
None declared.